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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

STUDIES COMPARING NUCLEIC ACID METABOLISM AND RESPIRATION IN PROTEUS VULGARIS, ITS STABLE L-PHASE AND THE SIMILAR PPLO

(Following is the translation of an article by O. Kandler, C. Zehender and J. Mueller, Botanic Institute, University of Munich, which appeared in the German language periodical Arch. for Microbiology, Vol. 24, 1956, pages 219-249. Translation performed by Constance L. Lust.)

The transformation of a bacterial cell into the L-form as discovered by Kleineberger (1935) and Dienes (1939, 1949), represents one of the most basic transformation of a cell that we know today. In extent it may be compared to the transformation of a normal tissue cell into a tumor cell, however it offers a more advantageous experimental approach in order to study details.

Many reports can be found about the morphological changes of a bacterial cell in the L-transformation (Dienes and Smith 1944, Stempen and Hutchinson 1951, Prittwitz and Gaffron 1953, Hopken and Bartmann 1955, Grasset 1955). All observations show a marked swelling up of cells, which is further connected with an increase in nuclear material. (Tulasne 1949, Schellenberg 1959, Kellenberger and Liebermeister 1955). The daughter organisms then bud out in all directions from these polynuclear large bodies (Hopken 1955). These cells then continue to exhibit a multipolarity in budding in their continuous multiplication.

The massive interference in the cellular mechanisms can of course also effect irreversible changes in the genetic substance under certain conditions, so that a return to the original bacterial form is no longer possible even after thousands of generations (even if the effect of the modifying agent, mostly penicillin, is withdrawn)(Kandler and Kandler 1956). However, other L-forms retain the ability to regenerate into the classical bacterial form after numerous penicillin passages. In this report a stable L-form was used exclusively, since it was considered to be at the most complete stage of transformation.

Contrary to morphological reports there is a paucity of work on physiological phenomenon on L-phases, even though it is undoubtably only knowledge of the physiological changes in the cell that will lead to a cause and effect understanding of the total process. In the respiration study concerning utilization of carbohydrates and the presence of oxidases no qualitative change was seen between bacteria and L-form of P. vulgaris (Kandler and Kandler 1955). The sensitivity of L-forms to penicillin and the loss of the ability to liquify gelatin and to growth on simple substrates does indicate a certain alteration of metabolism. Also Vandrely and Tulasne (1953) reported that the lipoid content of L-phase was much higher than in the bacterial form. These authors also reported that in three-week old L-forms of P. vulgaris the content of DNA is greater than RNA. This is contrary to the conditions of bacteria.

The many studies in recent years on nucleic acids (N.A.) of bacteria have shown that the total NA of the mass of the bacteria, as well as the relationship RNA/DNA is susceptible to variations depending on the state of development (Malmgreen and Heden 1947 a,b,c,d,e, Morse and Carter 1949, Belozersky 1947, Cohen 1947, Sherratt 1953, Wellerson 1958). Comparing the NA content of two different forms is only meaningful if one also has the protein content (+NA content) over the whole developmental cycle. Defined phases are not sufficient.

Often L-forms of bacteria are considered to be PPLO since they are both similar morphologically and also mulitply via multipolar budding (Kandler and Kandler 1954). Only one work exists now on the NA content of PPLO (Gerbers 1955). He reported total NA = 20%, where half is RNA or DNA. These determinations were only done on older cultures.

The intent of this report is to lay a groundwork on physiological bases for the L-phase transformation and the possible relationship between L-phase and PPLO. This will be done by measuring NA, protein and respiration intensity during growth of P. vulgaris, L-phase, and a strain of PPLO, were measured.

Materials and Methods

a) Organisms: Proteus strain Dienes 52 and a stable L-form isolated by Seiffert with the aid of penicillin. The L-form has been cultivated for over 150 passages on penicillin-free medium without reverting to bacterial form. It corresponds to type A, Dienes (1949) and Kandler and Kandler (1956). Strain "Findlay" (isolated by Findlay from mice) was the PPLO strain used.

b) Media and culture methods: All three organisms were grown on the same medium which Seiffert (personal communication) developed for PPLO: 0.7% peptone, 0.2% glucose, 0.5% yeast extract, 0.3% salt, 0.4% K_2HPO_4, 10% horse serum, pH 7.8. For PPLO and L-phase 1% agar was added, for Proteus 1.5%. Incubation was at 37°C, shaking cultures 27°C. It was better to add 0.8% phosphate salt for liquid cultures. In this solution the L-form also grew well. Originally it did not grow in liquid culture. Standard 500 ml bottles were used, for shaking cultures Fernbach flasks with 1.5 liters medium were used. These flasks worked especially well.

The stock cultures were transferred from plate to plate every 3-4 days. Transfer of PPLO and L-phase were always tested by streaking agar. The material for experiments was always grown in liquid media. Inoculation was accomplished either with agar pieces or with the steril organisms that were spun down from the 300 ml culture flask. The liquid media became cloudy with hot-sterilisation, so to avoid this they were all Seitz filtered under pressure.

c) Fractionation: Organisms were harvested in a high-speed centrifuge, 10 minutes at 12,000 x g; washed 2x with distilled water. P. vulgaris may be washed without losing cells, but L-phases and PPLO are more fragile and care must be exercized (Gerber 1955). As seen in table 1 no NA is lost with young PPLO; in older cultures up to 15% is lost. The RNA/DNA remains constant despite the losses. L-forms are already sensitive when they are young as is seen in the loss of NA (table 1). Because of this L-forms were usually not washed, but were used to fractionate right after centrifugation. In this way no total N was determined, since medium was still there. Also the first fraction for soluble N and P compounds was unsuitable.

NA were extracted as outlined by Heilinger (1954). A water soluble fraction is seperated and protein precipitated with TCA. After lipids are extracted NA are split with hot TCA or Perchloric acid, so that the smaller pieces are likewise soluble. The following general procedure was used.

Organisms were suspended in 10 ml water; 1 ml each for respiration, N, PO_4 (latter two in duplicate), other 5 ml and 0.5 ml 60% TCA, cooled and extracted 10 minutes, centrifuged, washed 2x with 6% TCA. Combined TCA extract one half each for PO_4 and N test precipitate was washed at 50°C with alcohol-ether to remove lipids. Ribose, deoxyribose and PO_4 and protein determinations were done. (see scheme outlined in figure 1).

d) Analytical procedures: 1) N-micro Kjeldahl (Klein 1932), 2) phosphate - Martland and Robinson (1928), 3) RNA - Hahn and Euler (1946), 4) DNA - According to Dische, 5) respiration - Warburg's manometric method was used, 6) suspension density - turbidity at 587 mm in 1 cm path.

7) Range of errors in measurements: The range of errors was estimated and calculated accroding to the usual statistics. The error spread is presented in table 3. Most errors were within a range of 3%; for the small TCA extracts and for lipids 15% DNA determination 6.8% range. The important ratio of RNA/DNA was in the very good range of 2.8%. These errors represent only errors in the methodology and not variations in the test materials. The ranges may be rather wide at times because of additional biological fluctuations. In the results to follow only typical data from a single experiment are used. An average value for seperate viruses is senseless since they are not a true collective in the statistical sense. For our results the curves are true and exact and meaningful (eg. RNA/DNA). An average value is presented (with range) in an accompanying table.

Results

1) Trials with normal forms of P. vulgaris

P. vulgaris was inoculated into cultures and incubated at 27°C

with shaking. After good turbidity appeared 100 ml was harvested. The values for the NA content and RNA/DNA ratios were the same in all controls. From day 2 they again remained constant. In order to detect the transition from stationary to log phase these cultures were not suited, and a heavier inoculum was made into a 1 liter culture. In a parallel set from the original culture NA and protein was measured. In the large inoculum cultures log phase is very short. Perhaps a part of the inoculated bacteria do not grow and provide some error here in the log phase. To circumvent this other flasks were inoculated with large transfers of log phase cultures. When stationary phase was reached a transfer was always done. In this way log phase was prolonged and NA maxima could be determined more accurately.

In table 4 the analytical results from a typical experiment are summarized. No significant cell mulitplication has occurred as the low increase in extinction points out. What was seen may be due to the growing (enlarging) of the cells already present. In this phase a marked NA increase occurrs, especially RNA, so that RNA/DNA reaches almost the maximal value. Tripling DNA content without the corresponding rise in cell number is in agreement with the concept that bacteria possess several nucleoides during this multiplication phase. Upon reaching stationary phase (4th hour) RNA content decreases in respect to DNA and protein.

No characteristic changes appear in these few fractions. Lipid-phosphate with respect to protein remains constant; TCA - soluble P a slow decrease in log phase; TCA - soluble N, however increases. This effect is not statistically of value because of a wide range. These fractions are not of importance in our later discussions. They were presented only to have a complete overview about the constituents of the fractions.

In numerous repetitions the maximal NA values deviated because of error in sampling time. The difference between log and stationary phase is undoubtably real as can be seen in the average value (with experimental ranges) of table 5.

A complete picture of changes in NA and protein and respiration during development is summarized in the results of figures 2-5. The first transfer came from a 50 ml, 48-hour old, resting culture. Three ml was taken out in a Warburg flask. From larger flasks 200 ml (later 50 ml) was removed every 50 minutes to measure O_2 uptake. Development was synchronous in the large and small flasks. Extinction and protein was measured. All growth curves were identical. Therefore, the O_2 utilisation could be used with respect to N and NA growth values.

Growth curves are presented in figures 2 and 3. The decrease of total growth in II and III is unexplained as yet. The effect was present in all parallel trials run.

Of particular interest are the quotients presented in figures 4 a-c. Run I and III had similar relationships of RNA/N as before,

whereas II increased even more. This maximum should characterize the particular state of P. vulgaris cells very well. DNA content also rises in II and reaches the same maximum in III. The rise in Q of DNA/ protein nitrogen is partly dependent on the size of the cell which decreases in long term studies. We repeatedly observed that in resting cultures (24 hours old) the size of the cell increased dramatically, whereas from transfers from stationary phase produced markedly smaller bacteria. Since the change in size affects the cytoplasm primarily RNA and protein and not nuclei, DNA the Q of DNA/protein increases and RNA/DNA decreases. This is well illustrated in figure 4c for trial III. Maximal values for RNA/DNA were seen in II. They are in the lag-early log phase where bacteria show the largest cells.

Absolute O_2 use of 10 ml suspension is shown in figure 5a. After a sharp rise with increasing cell multiplication, it remains constant briefly, and then decreases after log phase stops. O_2 use with respect to N moves the maximum to early log phase. The curves show a parallel to the RNA/N and decrease at the same time. The close dependence of RNA content and respiration-acitivity is again evident.

However, the respiration quotient does not increase in the same sense as RNA content. The Q O_2 use /RNA should remain relatively constant. As is shown in figure 5b this is not nearly the case. This curve also shows O_2 use per mg. DNA. They agree well with those curves that relate O_2 use and cell number (cell # from standard curve of extinction and growth). This is as expected, since nuclear mass can only deviate by a factor of 2 from the maximum and must remain constant (parallel) to the number of reproductive entities. Since in the organisms to be discussed later no counting of cells was possible we always used DNA content as a reference for size. The corresponding curves in Figure 5b show that the O_2 use of a bacteria reaches its maximum at the end of the lag phase at the transition to log phase. During rapid multiplication it decreases noticably. This is because substrates are exhausted, not due to degradation of enzyme systems.

2) Experiments with L-form of P. vulgaris

In the first experiments 10 bottles of 300 ml medium were inoculated with a grown-over piece of agar and incubated without shaking at 37°C. After good turbidity (24 hours) two bottles were centrifuged and the cells worked-up like P. vulgaris; no H_2O washing, medium was extracted in TCA wash. The other flasks with heavy growth were then used in one to several days.

After centrifuging 1 ml of 10 was used for oxygen uptake in a flask and after several minutes medium was added via the side arm. Respiration was constant after 1 hour and was used in the following series.

Before centrifuging the suspensions were studied to be sure they were bacteria free. The organisms are easily recognizable under phase

contrast. With young cultures many blisters appear which are clumped. A part appears strongly vacuolated and mostly empty - only on the virus do particles appear. No hemogeneous distribution was achieved, but with mechanical shaking the floculates do seperate into single organisms. Cell counts based on the usual dilution method are problematical. As their age grows the blisters become more highly vacuolated, the particles become smaller and hardly discernable. Upon streaking an agar many colonies were still formed. In figure 6a and b typical flocules are presented from young, still growing cultures. Figure 7 shows an older stationary culture. In still older cultures the microscopic picture is not changed appreciably.

In table 6 data on alterations in the cultures is presented. After 3 days the main growth of DNA and protein stops. In the period of the next week they only increase by two-fold. RNA decreases from the first test (24 hours). RNA seems to be degraded and used again in further growth. DNA and protein can however still be measured in the centrifugate.

The Q values also show a typical pattern corresponding to the absolute patterns. RNA/DNA and RNA/protein N decrease rapidly and reach very low levels; lower than in even very old bacterial cultures. The RNA DNA relationship is even the reverse in the old suspensions. In all samples tested values under 1 were observed. This agrees with the findings of Vendrely and Tulasne (1953) who also used old cultures. Our studies showed that this is not a typical property of L-forms, because in the vegetative state the value is near 5; in the range of bacteria even though in P. vulgaris values are 2 times that. It is questionable that the values for RNA/protein and RNA/DNA of old cultures are representative for the resting forms of L-forms. Cell counts showed that the numbers of colony forming elements decreased by a factor of ten. The single organisms generally are clustered in groups so that each colony perhaps represents many entities. If a number of cells die in these groups the rest still form a colony and mark the regression of cell number. It is possible that in cultures with a decreased cell count only a few are viable and in analyzing the tissue constituents only the part that did not become soluble (from dead cells) was measured.

To answer this aspect measurement of intensity of respiration is desirable. If one gives new substrates to the resting organisms the O_2 use will show how active the enzyme systems are in the cell. As table 6 shows the intensity of respiration with respect to protein remains constant over 14d. This also holds for O_2 use/DNA which may be thought of as O_2/cell. An increase of 20% in the first 8 d is apparent. This means that the marked decrease of RNA is no indication of the destruction of the cell, because the main decrease appears in the first 14d during which respiration is still increasing. Accordingly the O_2 use/RNA increase in this time RNA degradation and lowering of RNA/DNA toward one represents a normal process in L-forms and is not indicative of inactivation.

Whether the further decrease of NA content is normal remains questionable, since respiration is much reduced in these old cultures. This may not be evidence for cell death, because it could be that the resting L-forms degrade their enzymes and then regenerate in a long lag phase, so that observation time only minute respiration may be seen. In longer observation periods the respiration goes up, but it is difficult to say whether all cells increase or if only some duplicate and this is then measured. The NA content question is resting L-forms remains unanswered for now. It can only be stated that old suspensions have a very low Q for RNA/protein and RNA/DNA.

It appeared of importance to understand the transition from resting cultures to the growth and reproductive phase. Large inocula were used so that measurable growth and contents appeared in short time. The results of such an experiment appear in table 7. RNA is degraded right after stop of log phase, whereas protein increases more and DNA remains constant.

The way in which the values for RNA/protein run points clearly to the fact that RNA synthesis preceeds protein synthesis. The values for DNA/protein increase and reach a max in the middle of log phase. RNA/DNA is maximal in lag phase or early log phase. Table 8 contains data on NA/protein for log and early stationary phase for 8 samples.

As with P. vulgaris, L-forms in two runs were given new medium in early log phase; and once after stationary phase. Figure 8 a-g contain the results. The exact curve allows a detailed discussion. The same characteristic changes as seen earlier were present. Even when log phase was prolonged no increase in Q NA/protein or RNA/DNA was seen. It should be noted that the pH changes may be important. This was also seen before. The glucose containing substrate first gets acidic as in P. vulgaris cultures, but after multiplication it turns alkaline. The latter was not seen previously.

Oxygen consumption was measured here the same way as in the case for the P. vulgaris experiments. Figure 8f shows the absolute O_2 use per 3 ml suspension per hour. It reached a max toward the end of log phase. O_2/protein reaches a max earlier. The stop of respiratory intensity is due to lack of substrate. These curves should not be compared to the curves in table 6, since the new substrate was added and respiration was measured over 2 hours. The N content of the suspension was about 10x higher there. Under these conditions practically no growth occurrs evidently because of inhibition due to end products and lower pH. Respiration intensity is markedly less here than in growing cultures. This was also true for bacteria.

Respiration with respect to RNA shows a different picture for L-forms than for Proteus (figure 8g). The horizontal pattern of the curves shows that proportionality is present for a long period. O_2/DNA also show a different pattern than in Proteus. The change of respiration per viable cell apparently runs smoother and slower.

Max is in the early log phase. Respiration velocities for L-forms are lower than those of Proteus. This corresponds to the slower development. Materials are used slowly in the stationary phase. In this period oxydative metabolism reaches a maximum.

3) Experiments with PPLO

Similar to L-forms a turbidity appears about 24 hours after inoculation with a piece of agar in liquid cultures of PPLO. Microscopic studies in phase contrast showed large flocular forms which may represent small colonies. On their edge small coccal entities are recognizable (figure 9a). After 24 hours more, with shaking, these forms dissolve again into smaller groups. (figure 9b). It is difficult to show this photographically because they exhibit Brownian motion. Comparing the two figures one readily sees the differences. Similar phenomenon appear in cultures that are shaken. Even here homogeneous cultures do not appear as in bacteria. Turbid forms appear from which the individual seem to be formed; are evolved from the membranes.

It is just as difficult to determine cell counts with the dilution technique with PPLO as with L-forms. The count creeps higher with time and age and mechanical upset because of release of more single entities. We found that the counts varied greatly in growing cultures. Generally we found a continous rise based on extinction and absolute increases in protein and NA.

In several experiments we also used electron microscopic studies parallel to NA determinations. The preparations were dried from a washed suspension and placed on carrier grids. The objects were placed in T_2O_2 vapors. The same pictures came out as previously (Kandler et al 1954). The measurements are presented in table 9. With age and decreasing RNA the diameter of the particles decreased.

In table 10 results are presented about cultures that were inoculated with low inocula. As in L-forms RNA decreases at a time when protein still increases. DNA content remains constant over a long period. RNA/DNA is between 3 and 3.8 for young cultures, and 1.5 - 2.0 in older ones. Even in very old cultures it doesn't sink below 1.0 (as in L-phases).

The values for respiration intensity decrease more rapidly than was the case for L-forms. Respiration per DNA remains constant for 5 days. During this time RNA content decreases sharply. It may be concluded then that degradation of RNA (as in L-forms) is a normal process and not a sign of the impending death of the organism.

It cannot be differentiated whether the analytical data of older suspensions are representative of resting, but living cells, or if it represents only those of dead organisms. Undoubtedly a part of the cells of liquid cultures is viable for long periods of time. Seiffert (personnal communication) could inoculate PPLO strain successfully which had been stored in sealed ampules for 10 years.

The transition of stationary -to log-phase was again studied in 11 sets with large inocula of various ages (1-14d). Table 11 contains absolute values of quotients. Development occurrs in 24 hours. During lag phase, when extinction increases minumally, RNA reaches a max and begins to decrease soon thereafter. Contrary to L-forms DNA/protein remains high and decreases only in later stationary phase. Average values for RNA/DNA and NA/protein for all experiments are summarized in table 12.

The relationship between NA content and max oxydative metabolism during multiplication of PPLO was studied with repeated inoculations. At first a 48 hour liquid culture was used. The results are shown in figure 10a-c.

At the end of an 8 hour lag phase RNA/protein and RNA/DNA reach maxima. During log phase both values are lower. In III, which was inoculated from stationary phase an increase appeared later. The transition from resting form to the viable vegetative state appears to be associated with a marked elevation of RNA. During rapid multiplication the RNA content decreases, eg. low values of RNA/protein and RNA/DNA in II. This is contrary to Proteus and its L-form, where in a prolonged log phase the maxima remained up for longer times. The O_2 use also deviates from the conditions for the other organisms. Maxima for O_2 use are in the early log phase, and yet respiration decreases at this time. All curves, therefore, show a decreased slope in the period of main reproduction. The max of oxydative metabolism corresponds to that of RNA increase. The velocity with which O_2 is used in PPLO is decidedly less than for Proteus.

Discussion of Results

From the foregoing data one can compare the metabolic changes during development of three different organisms-forms. For this purpose all curves of NA metabolism are combined from the three organisims. The time axis was altered so that the points of a curve for the same development stage were superimposed. Presented is always a typical curve from a large inoculum from early stationary phase. In figure 11c the extinction curve is also presented, in order to have a frame of reference. Results of older cultures are not included, since there is doubt about whether these are viable cells or dead cells.

In figure 11a the Q of DNA/protein is shown. It shows that L-forms in all developmental stages is about 25% richer in DNA than the bacteria. The slope of the curve is practically equal. PPLO contains the same DNA content as L-forms, but the curves are different. The decrease slows after growth ceases.

Greater differences between the three organisms exist for RNA content (figure 11b). L-forms contain about 50% less than bacteria. The curves appear similar RNA in transition from stationary to log phase is about 300%. PPLO, however only has 50%, and is 50% less than L-form. The greatest alterations appear in RNA/DNA (figure 11c).

L-form and bacteria are different. The PPLO curve is very much flatter and max is at the end of log phase. The disappearance of RNA even during multiplication appears to be a very characteristic marking of PPLO. Up to now limited data were available in RNA/DNA for bacteria and particularly so expressed in terms of alterations during growth. Nevertheless it may be assumed that such a marked reduction of RNA does not occur in bacteria also see Rippel and Burch 1954.

Another difference appears in comparing lipid-phosphate. In table 13 results are seen of phosphate/protein. L-phase contains 2x the phosphate as bacteria, while PPLO have the lowest amount. Vendreley and Tulanse (1953) also reported a very high lipid content in L-forms.

When RNA diminishes respiration behaves in a paralled fashion. Comparing O_2 per protein or DNA during development (table 4) shows that Proteus decreases to 1/3 in PPLO. This does not mean that oxydative metabolism for synthesis also decreases. This may be seen if one calculates how much O_2 is used when 1 mg N is assimilated from the medium. This calculation can be easily done, in those experiments where O_2 use and synthetic increase were both measured. Table 15 contains corresponding data. They are calculated for two sets; lag phase - log phase and total growth from beginning to end of log phase (no stationary).

First it may be seen, surprisingly, that Proteus and PPLO use the same O_2 in the synthesis of equivelent quantities of cell materials. In both it is greater early than in total growth. L-form also has an elevated O_2-need early, but not so great as bacteria during multiplication, as may be seen in the table.

Summarizing the results of these studies one can say:

1) Transformation of P. vulgaris into the stable L-form is associated with a strong quantitative change of NA metabolism. RNA/DNA decreases by 50% but shows the same principle variations during development as in the bacteria. DNA content is elevated and the decrease of total NA is due to the decrease of RNA.

2) L-forms utilize O_2 30% slower, but the percentage of Oxydative metabolism needed for syntheses is not noticeably different because growth velocity is correspondly slowed.

3) PPLO exhibit elevated DNA as do the L-phases. RNA is decreased less and Q RNA/DNA has less variations during development. In this way they differ significantly from bacteria and L-phases. Unfortunately there is a lack of data on NA constitution of pathological animal viruses, which contain mainly DNA but should also have RNA (Schramm 1954). It may be that PPLO are intermediate in NA content between bacteria and the large viruses (Rusha and Poppe 1947, Edward 1954)

The marked quantitative changes in NA metabolism may signify that the transformation into L-phase also has accompanying qualitative changes

in NA. A study of this aspect would undoubtedly add significant information to these still puzzling phenomena.

Summary

Proteus vulgaris, its stable L-form and a strain of PPLO during development were studied as to the content of DNA, RNA and protein. Simultaneously oxygen consumption was measured.

For P. vulgaris the max for NA content and Q RNA/DNA fall in the early log phase; Q DNA/protein max is between log -+ stationary phase. These values differ by only 50%-300%. Maximum O_2 use in early log phase is 5000 mm^3 per mg protein-N per hour. In order to sustain 1 mg N of bacterial mass 3200 mm^3 O_2 are used during the growth period.

L-phase as a 25% increased DNA content, whereas RNA is 50% less than in bacteria. RNA/DNA shows the same trend during growth as for bacteria but is 50% lower than in P. vulgaris. Maximum respiration is 1800 mm^3/mg protein per hour. During the assimilation of 1 mg total N 5400 mm^3 O_2 are utilized.

PPLO are different from the other two forms in having markedly less RNA which varies only 30% during development. DNA content is the same as for L-forms. Respiration intensity is 600 mm^3 per mg N per hour (very low), but the O_2-use during assimilation of 1 mg total N (or DNA) is (as in P. vulgaris) 4500-8000 mm^3. Under aerobic conditions oxydative metabolism is just as important for synthesis as for P. vulgaris.

Legend for words in tables and figures

DNS = DNA; RNS = RNA; eiweiss = protein; atmung = respiration; lipoid = lipid; ges = total; TEI = TCA; NS = nucleic acid (NA); werte = values; zeit = time

Table 1

Nucleic acid (NA) content of 2-14d old PPLO after repeated washing

	2 Tage alt			14 Tage alt		
Zahl der Auswaschungen	0	1	2	0	1	2
RNS [γ]	325	316	310	136	118	104
DNS [γ]	169	166	164	71,5	60	54
RNS/DNS	2,11	2,12	2,11	1,9	1,96	1,93
$\frac{-mm^3\ O_2}{1\ ml\ Susp.\cdot Std}$	217	210	204	—	—	—

Table 2

NA content of 4d old L-forms after repeated washing

Zahl der Auswaschungen	0	1	2
RNS [γ]	226	212	172
DNS [γ]	116	106	79
RNS/DNS . . .	2,00	2,00	2,18
$\frac{-mm^3\ O_2}{1\ ml\ Susp.\cdot 60\ min}$	189	137	110

Table 3

	N ges. [γ]	PO$_4^{3-}$ges. [γ]	PO$_4^{3-}$TES [γ]	PO$_4^{3-}$Lipoid [γ]	PO$_4^{3-}$Nucl.s. [γ]	DNS [γ]	RNS [γ]	N TES [γ]	N Eiweiß [γ]
Mittel-werte	1022	350	32	28	288	323	1083	84	714
σ . .	± 25	± 9	± 3	± 3	± 4	±22	±16	± 10	± 17

Tab. 3 (Fortsetzung)

PO$_4^{3-}$ges. Eiweiß-N [γ/mg]	PO$_4^{3-}$TES Eiweiß-N [γ/mg]	PO$_4^{3-}$Lipoid Eiweiß-N [γ/mg]	PO$_4^{3-}$Nucl.s. Eiweiß-N [γ/mg]	DNS Eiweiß-N [γ/mg]	RNS Eiweiß-N [γ/mg]	RNS DNS	N TES Eiweiß-N [γ/mg]	Extraktion
490	59	36	403	448	1510	3,40	117	50,2
± 24	± 4	± 4	± 17	± 33	± 56	±0,094	± 15	± 0,2

Overview of error-range of the quantitative values in single fractions

(Fortsetzung = continuation)

Zeit [h]	Extinktion	N ges. [γ]	PO$_4^{3-}$ges. [γ]	PO$_4^{3-}$TES [γ]	PO$_4^{3-}$Lipoid [γ]	PO$_4^{3-}$Nucl.s. [γ]	DNS [γ]	RNS [γ]	N TES [γ]
0	6,9	44	18	3	2	15	13	55	3
0,5	9,4	123	62	6	5	55	36	213	13
1,5	18,5	357	198	14	14	170	89	695	27
2,5	33,0	740	382	32	29	346	172	1370	50
4,0	55,9	1400	688	80	58	543	324	1960	110
6,5	74,4	2220	985	179	99	733	603	2525	170

Tab. 4 (Fortsetzung)

N Eiweiß [γ]	PO$_4^{3-}$ges. Eiweiß-N [γ/mg]	PO$_4^{3-}$TES Eiweiß-N [γ/mg]	PO$_4^{3-}$Lip. Eiweiß-N [γ/mg]	PO$_4^{3-}$Nucl.s. Eiweiß-N [γ/mg]	DNS Eiweiß-N [γ/mg]	RNS Eiweiß-N [γ/mg]	RNS / DNS	N TES Eiweiß-N [γ/mg]
30	594	102	68	509	426	1870	4,4	102
74	838	81	67	750	486	2010	5,9	179
200	986	67	67	846	444	3460	7,8	132
434	880	73	67	706	305	3160	8,0	115
912	754	87	63	505	356	2150	6,05	121
1531	643	117	65	478	394	1650	4,2	111

Table 4

Overview of alterations of metabolic make-up of P. vulgaris during growth

	RNS/DNS	RNS/DNS	RNS Eiweiß-N [γ/mg]	RNS Eiweiß-N [γ/mg]	DNS Eiweiß-N [γ/mg]	DNS Eiweiß-N [γ/mg]
Maximal-werte	7,28	± 1,01	312	± 34	46,2	± 10,6
stat. Phase	3,93	± 0,30	180	± 34	43,7	± 10,5

Table 5

Average values for NA content of P. vulgaris during log-stationary phase n = 12

Kultur-dauer in d	Eiweiß-N γ/10 ml	RNS γ/10 ml	DNS γ/10 ml	RNS Eiweiß-N γ/mg	DNS Eiweiß-N γ/mg	RNS DNS	$mm^3 O_2$ 10 min · mg Eiweiß-N	$mm^3 O_2$ 10 min · mg RNS	$mm^3 O_2$ 10 min · mg DNS
1	230	740	140	264	500	5,28	44	15	90
3	340	530	185	1550	544	2,92	52	33	95
4	360	470	200	1305	555	2,35	60	46	103
6	390	380	215	980	552	1,77	68	68	115
13	430	280	240	570	490	1,16	55	97	112
24	620	180	255	290	412	0,73	23	82	58
44	650	160	260	243	395	0,67	4	17	12

Table 6

Overview on the changes in NA content and respiration intensity of L-phases in a large culture

Kulturdauer in h	Extinktion	Eiweiß-N γ/10 ml	RNS γ/10 ml	DNS γ/10 ml	RNS Eiweiß-N γ/mg	DNS Eiweiß-N γ/mg	RNS DNS
0	0,045	30	18	12	600	400	1,5
13	0,06	150	300	75	2000	500	4,0
15,5	0,125	220	530	110	2420	500	4,8
17,5	0,210	285	770	160	2900	603	4,82
19,5	0,280	365	1006	240	2740	658	4,17
21,5	0,35	530	1300	310	2500	585	4,2
23,5	0,365	580	1110	320	1920	552	3,5
25,5	0,375	620	1040	310	1680	500	3,35
39	0,440	830	710	290	855	350	2,45
46	0,460	880	690	280	782	318	2,46

Table 7

Summary of changes of NA during growth in a large inoculum

	RNS/DNS	RNS/DNS	RNS Eiweiß-N	γ/mg	RNS Eiweiß-N	γ/mg	DNS Eiweiß-N	γ/mg	DNS Eiweiß-N	γ/mg
Maximal-werte	5,40	± 0,75	2300		± 615		630		± 108	
stat. Phase	1,70	± 0,30	870		± 130		490		± 70	

Table 8

Average values of Q's in order to characterize NA of P. vulgaris

Zeit	Kolloidaler Durchmesser (mμ)	σ (mμ)	n
24 h	0,70	± 0,035	30
36 h	0,53	± 0,031	30
4 d	0,44	± 0,028	30
5 d	0,35	± 0,013	30
12 d	0,34	± 0,024	30
26 d	0,32	± 0,018	30

Table 9

Average value of diameters of particles of PPLO cultures

Kulturdauer	Eiweiß-N γ/10 ml	RNS γ/10 ml	DNS γ/10 ml	RNS Eiweiß-N γ/mg	DNS Eiweiß-N γ/mg	RNS DNS	$mm^3 O_2$ 10 min · mg Eiweiß-N	$mm^3 O_2$ 10 min · mg RNS	$mm^3 O_2$ 10 min · mg DNS
20 h	51	92	23	1800	490	3,68	22	14	45
36 h	120	185	48	1540	400	3,85	21	13	50
4 d	184	122	38	660	207	3,2	13	19	61
5 d	178	98	35	550	197	2,8	11	20	54
12 d	160	61	28	380	175	2,18.	4	13	26
26 d	138	46	24	333	174	1,91	1	2	3

Table 10

Summary on NA and respiration of PPLO in various cultures

Kulturdauer in h	Extinktion	Eiweiß-N γ/10 ml	RNS γ/10 ml	DNS γ/10 ml	RNS / Eiweiß-N γ/mg	DNS / Eiweiß-N γ/mg	RNS / DNS
0	0,018	24	14	8	582	333	1,75
13,5	0,035	46	93	32	2006	692	2,97
16	0,038	55	110	38	2000	692	2,9
18	0,042	70	134	46	1920	660	2,92
21	0,052	108	184	72	1715	670	2,55
23	0,064	132	212	88	1605	650	2,43
26	0,093	—	—	—	—	—	—
37	0,265	238	238	158	1008	660	1,70

Table 11

NA content of PPLO in dependence of growth on a large inoculum

	RNS/DNS	σ RNS/DNS	RNS Eiweiß-N γ/mg	σ RNS Eiweiß-N γ/mg	DNS Eiweiß-N γ/mg	σ DNS Eiweiß-N γ/mg
Maximalwerte	3,41	± 0,63	1850	± 270	680	± 133
stat. Phase	1,80	± 0,32	1110	± 140	620	± 83

Table 12

Average values of NA of PPLO at start of log and stationary phase
n = 11

Organismen	Lipid-Substanzen PO_4^{---}/mg Ges.-N
Proteus vulgaris .	65
Stabile L-Phase . .	120
PPLO (Stamm Findlay)	40

Table 13 - Average content of lipid-PO_4 with respect to 1 mg protein-N

Organismen	$-O_2$ / mg Eiweiß-N · h	$-O_2$ / mg DNS · h
Proteus vulgaris .	5000	11000
Stabile L-Phase .	1800	3000
PPLO(Stamm Findlay)	600	900

Table 14

Comparison of maximal respiration in 3 organisims per 1 mg N or 1 mg DNA.

Organismen	Wachstum bis z. frühen log-Phase		Wachstum bis zur stat. Phase	
	$-O_2$/mg Ges.-N	$-O_2$/mg DNS	$-O_2$/mg Ges.-N	$-O_2$/mg DNS
Proteus vulg. . .	4200	17000	3200	10000
Stabile L-Phase	9500	36000	5400	12000
PPLO (Stamm Findlay) . . .	4500	8700	3500	8000

Table 15

Oxygen consumption during synthesis of 1 mg DNA and/or protein from 1 mg N of cell substance.

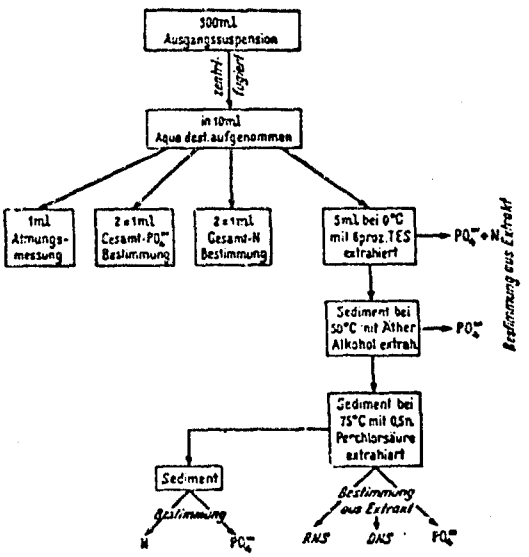
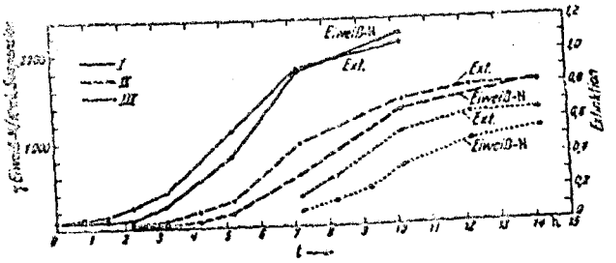


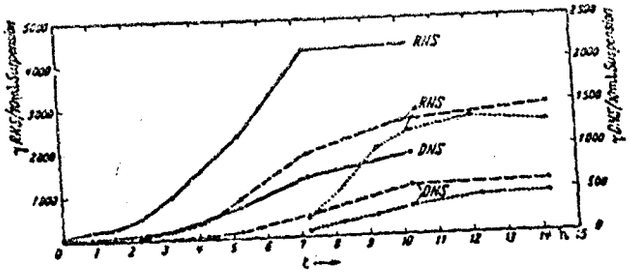
Figure 1 - Schema for fractionation procedures (see methods)

Figure 2



Increase of entinction and protein - N per 10 ml. suspension with repeated inoculation of P. vulgaris

Figure 3



Increase of RNA and DNA

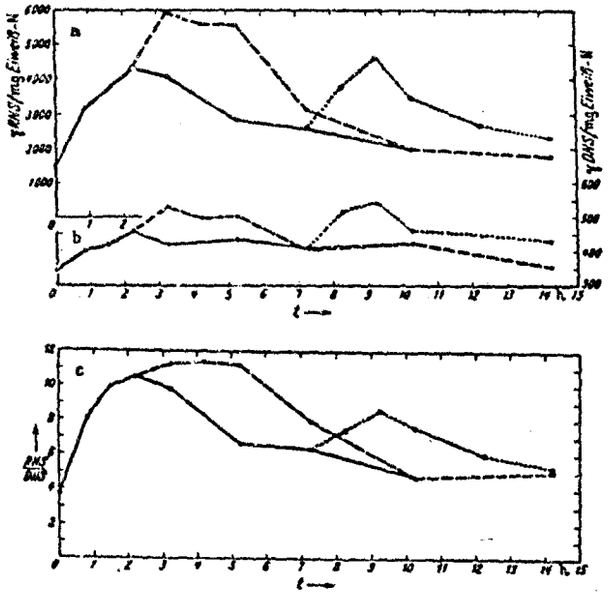


Figure 4 a-c - Dependence of Q RNA/protein -N; DNA/protein N and RNA/DNA

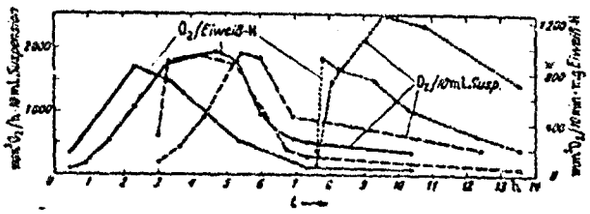


Figure 5a
Respiration velocity dependence on growth (per mg Protein N)

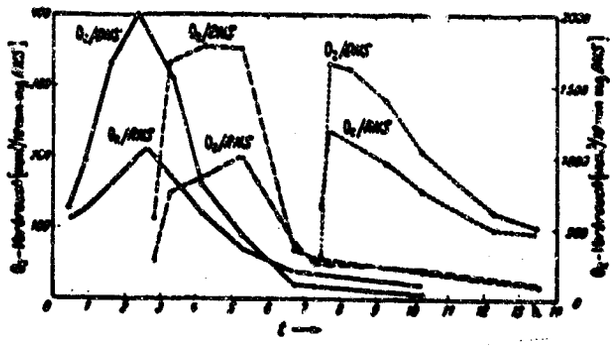


Figure 5b - As for figure 5a per mg RNA and DNA

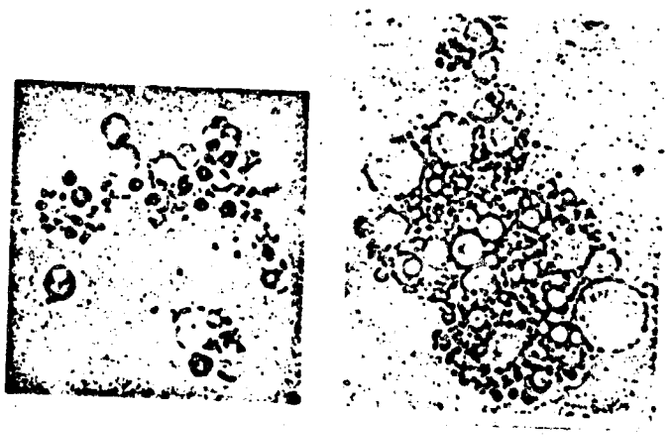


Figure 6a-b - Single organisims and "grape" clusters in 2d old cultures of protein L-form (phase contrast 800x)

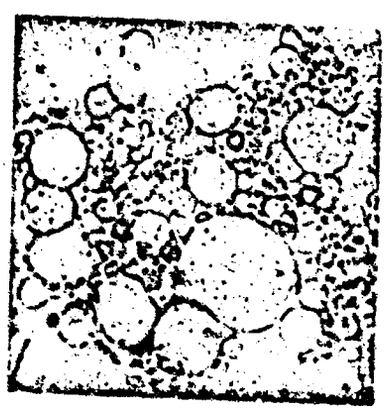


Figure 7 - Typical floculation in culture (4d) of L-form (800x)

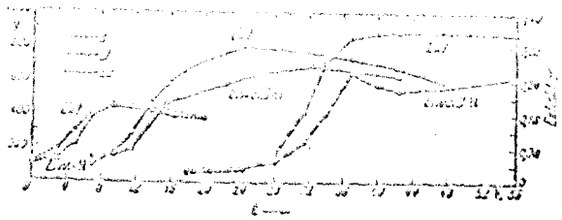


Figure 8a - Growth of L-forms (extinction and protein-N per 10 ml

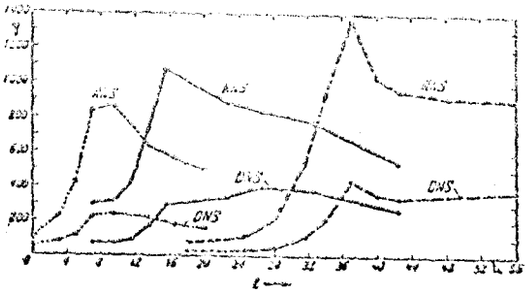


Figure 8b - RNA and DNA increase per 10 ml

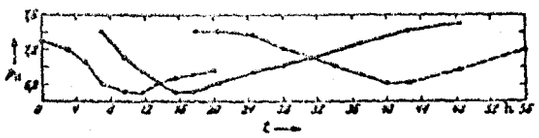


Figure 8c - pH changes in medium

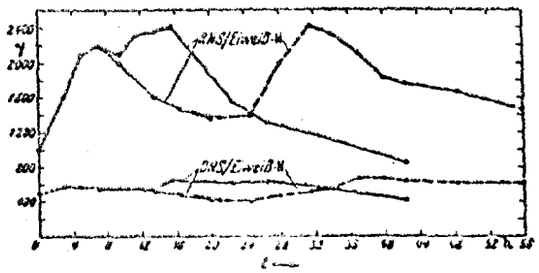


Figure 8d - Change of Q RNA/protein N and DNA/N

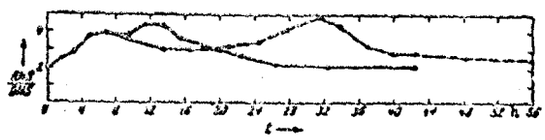


Figure 8e - Changes in RNA/DNA

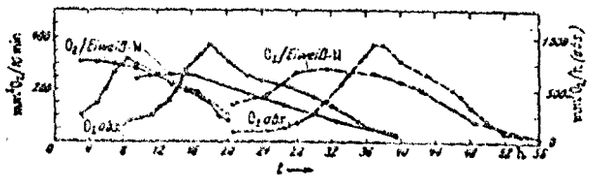


Figure 8f - Dependence of respiration on growth (10 ml or 1 mg protein N)

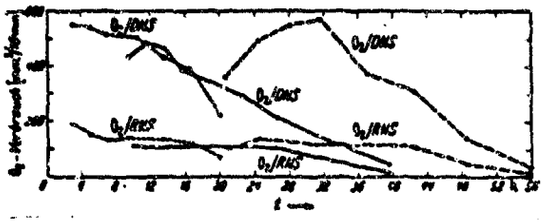


Figure 8g - eg 8f per 1 mg DNA and RNA

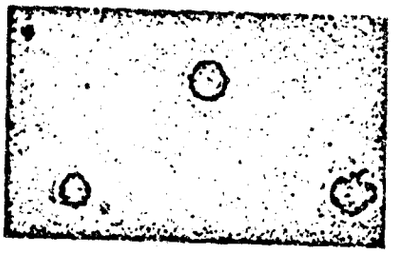


Figure 9a - forms of PPLO in cultures (24 hrs) phase contrast 800x

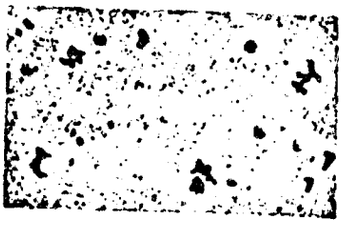


Figure 9b - eg. 9a 24 hours later 800x

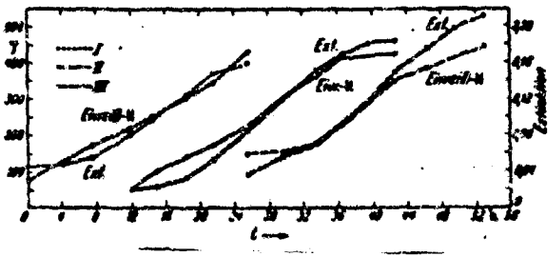


Figure 10a - growth of PPLO extinction and protein N

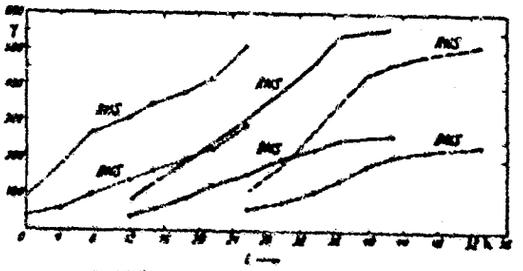


Figure 10b - Increase in DNA and RNA

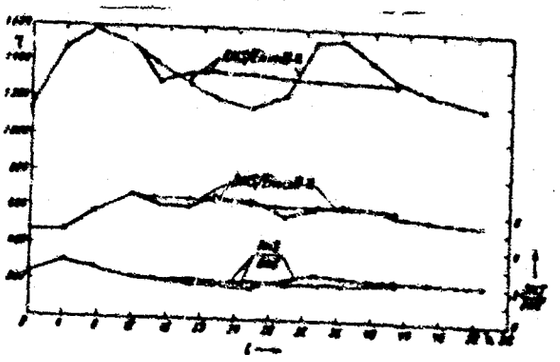


Figure 10c - Quotients of 10 a and b

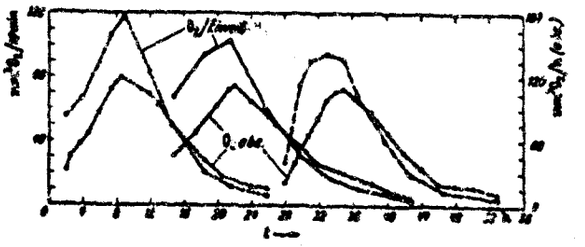


Figure 10d - Respiration during growth per 10 ml and mg protein N

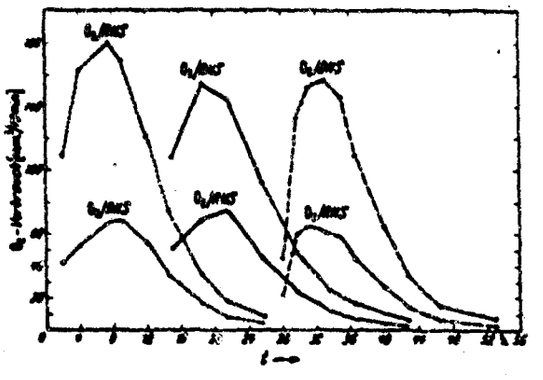


Figure 10e - As 10d per day DNA and RNA

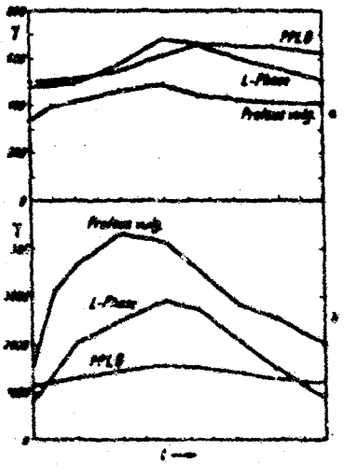


Figure 11a - Comparison of Q DNA/protein of 3 organisms

Figure 11b - as. 11a for RNA/protein

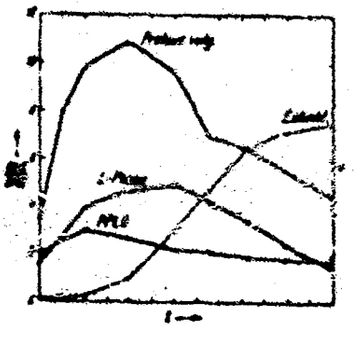


Figure 11c - as 11a for RNA/DNA

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